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Identification of Malassezia Species in Seborrheic Dermatitis Patients Using Nested-pcr

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Abstract

Seborrheic Dermatitis is a common chronic papulosquamous disease and may affect adults and infants. This study aims to determine the frequency of *Malassezia* species among Seborrheic dermatitis patients using Nested-PCR; and to evaluate the effect of HIV and non-HIV on the incidence of *Malassezia* species. The research was conducted at Dr. Wahidin Sudirohusodo hospital and other hospitals in Makassar from June 2017 to August 2017. This study used analytic and descriptive observational method with cross sectional approach. The samples were Seborrheic dermatitis patients aged over 18 years old who came to dermatovenereology outpatient department of Dr. Wahidin Sudirohusodo Hospital and other hospital in Makassar. A total of 30 samples of seborrheic dermatitis patients, consisting of 5 HIV positive and 25 non-HIV patients, were scraped to collect the Seborrheic specimens, then Nested-PCR was performed to identify the *Malassezia* species. The results showed a high frequency of *Malassezia* species in seborrheic dermatitis patients, of which 30 samples obtained 23 samples was positive and 7 samples was negative, with *Malassezia restricta* as dominant species. Using nested PCR, of the 5 HIV positive patients, 4 samples (80%) were positive for *Malassezia*, while from 25 samples of non-HIV patients 19 (76%) of them were positive for *Malassezia*.

Keywords: Seborrheic Dermatitis; Nested-PCR.

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1. Introduction

Seborrheic dermatitis is a common chronic papulosquamous disease and may affect adults and infants. Characteristically found in areas of the body with high concentrations of sebaceous follicles and active sebaceous glands, including the face, scalp, upper part of the body, and the intertriginous area (inguinal, infrared, and axilla). Clinical features appear as pink to erythema, plaque and superficial spots with yellowish scales, and sometimes oily. Seborrheic dermatitis is one of the most common forms of dermatosis in patients with Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) as well as in neurological disorders, such as Parkinson's disease [1].

In HIV-infected patients, seborrheic dermatitis is often more inflamed and severe than normal individuals. The onset of seborrhoeic dermatitis in HIV patients is very rapid and rash can be very widespread. Skin disease causes significant morbidity and can generally be an early symptom of an immunosuppression. Skin manifestations have been shown to be a valuable clinical indicator of HIV infection and a linkage of some skin conditions to CD4 + T cell counts to individuals with HIV has been established. Several studies have shown an association between skin disorders with HIV infection, immunosuppression and decreased CD4 + cell count [2,3].

The pathogenesis of seborrhoeic dermatitis has not been fully explained, but is associated with *Malassezia* fungi, immunological abnormalities, glandular activity and individual susceptibility. Many patients have normal amount of *Malassezia* on the skin, but they have an abnormal immune response, resulting in suppression of T-helper cell response. *Malassezia sp*. also plays an important role in the inflammatory response with stimulation of alternative complementary pathways. Disturbance in the lymphocytic cellular immune response to *Malassezia* results in increased interleukin (IL) -10 as well as a decrease in IL-2 and interferon-γ [1].

Several clinical studies have shown increased *Malassezia* density has an important role in the pathogenesis of seborrhoeic dermatitis [4]. *Malassezia* is a lipophilic dimorphic fungus belonging to normal flora and can be isolated from skin scrapings that originate from almost all areas of the body, especially in areas rich in sebaceous glands such as the chest, back and head area. It can be found in 90% of healthy adult skin and can change the state of saprophyte into a pathogen under the influence of predisposing factors, such as changes in skin microflora and changes in host defense [5].

Nested-PCR is a DNA samples replication technique using DNA polymerase enzymes that use two primary pairs to amplify fragments. By using Nested-PCR, if any erroneous in amplifiving fragment then it is likely to be amplified second time by a second primer. Thus, Nested-PCR is a very specific PCR in amplifying. Lim and his colleagues used Nested-PCR in previous research to identify *Malassezia* species in patients with seborrheic dermatitis [6]. A recent study in Ahvaz, Iran, identified the dominant species in patients with pityriasis versicolor and seborrhoeic dermatitis with Nested-PCR test, and the result of the dominant *Malassezia* species was *M. furfur* followed by *M. restricta* [7].

Based on the above research, the authors are interested in conducting a study aimed at determining the

frequency of *Malassezia* species found Seborrhoeic dermatitis patients using Nested-PCR and evaluate the effect of HIV and non-HIV status on the incidence of *Malassezia* species.

2. Material and Methods

Time and Place

The study was conducted in the Dermatovenereology Department of Dr. Wahidin Sudirohusodo Hospital, and networking hospital in Makassar. Nested-PCR was performed at the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University. The research was conducted in June to August 2017, for 3 months until the sample was collected.

Design Study and Variable

This study used observational analytic and descriptive method with cross-sectional approach. The variables consisted of: independent variables (*Malassezia* species, HIV AIDS, other infections, drugs, genetics, age, sex), dependent variables (Seborrheic dermatitis), and intermediate variables (*Malassezia*'s role in the pathogenesis of Seborrheic dermatitis).

Population and Sample

Patients with Seborrheic dermatitis with both HIV and non-HIV, who came to the outpatient department of Dr. Wahidin Sudirohusodo Hospital and other networking hospital in Makassar. All study sample was the entire population that met the inclusion criteria.

Data Collection

Patients who met the inclusion and exclusion criteria and were willing to contribute were asked to sign an informed consent. Afterwards, the samples was divided into 2 groups based on HIV status. Then, a seborrheic scraping specimen was taken using a blunt scalpel blade to obtain the scales. Scales is contained on a object glass that has been fixated. The specimen then was isolated and prepared for Nested-PCR.

Data Analysis

The data was processed using SPSS version 18. Univariate analysis was performed to test the hypothesis in determining the frequency of *Malassezia*, while Chi-square test was used to analyze the influence of HIV and non-HIV against *Malassezia*. The significance level used is $P < 0.05$ with a 95% confidence interval.

3. Results

Analytical and descriptive observational studies have been conducted with cross-sectional approach to determine the frequency of *Malassezia* species in seborrheic dermatitis patients using Nested-PCR, and to evaluate the effect of HIV and non-HIV on the incidence of *Malassezia* species. This study was conducted with

samples who met the inclusion criteria in Dr. Wahidin Sudirohusodo Hospital and other networking hospitals in Makassar, South Sulawesi. The sample consisted of 30 Seborrheic dermatitis patients consisting of 5 HIV positive and 25 non-HIV patients.

From the total samples, 17 subjects (56.7%) were male and 13 subjects (43.3%) were female. The age group of 41-70 years had the largest amount of sample whic consisted of 20 people (66.7%), while the lowest was 10 samples in the 18-40 years age group (33.3%). Based on the family history, 3 subjects (10%) were positive and 27 others (90%) had no family history of seborrheic dermatitis. From 30 samples, 23 (76,7%) showed positive PCR test result and 7 samples (23,3%) were negative (attachment, Table 1).

Table 1: Subjects Characteristics

Category	Frequency (n)	Percentage (%)
Sex		
Male	17	56.7
Female	13	43.3
Age		
18 – 40 years	10	33.3
41 – 70 years	20	66.7
Occupation		
Employed	23	76.7
Unemployed	7	23.3
Family History		
Positive	3	10.0
Negative	27	90.0
PCR		
Positive	23	76.7
Negative	7	23.3
Total	30	100

Note : PCR = *Polymerase chain reaction*

The frequency of *Malassezia* species in Seborrheic dermatitis patients with HIV and non-HIV showed that in 5 patients with Seborrheic dermatitis and HIV, there were 4 (80%) of them were PCR positive and 1 sample (20%) was negative.

Whereas in patients with non-HIV Seborrheic dermatitis there were 19 samples (76%) with positive PCR and 6 samples (24%) were negative. Thus, the frequency of *Malassezia* species found in Seborrheic dermatitis patients with HIV was 80% and in non-HIV Seborrheic dermatitis patients was 76% (annex, Table 2).

The relation of HIV status to *Malassezia* species in this study showed positive PCR results in 4 samples (13.3%) of HIV patients and 19 samples (63.3%) of non-HIV patients.

Negative PCR results were obtained in 1 sample (3.3%) of HIV patients and 6 samples (20%) of non-HIV patients. Overall, total PCR positive results were 23 samples (76.7%), and negative PCR results were 7 samples (23.3%). There was no significant difference in the incidence of *Malassezia* species between HIV and non-HIV patients, with P = 1,000 (attachment, Table 3).

Electrophoresis of PCR products in the sample group of Seborrheic dermatitis patients showed positive predominance of *M. restricta* on target band of 320 bp with forward primer CTTGGTTGGACCGTCACTG and reverse primer AGGCGGATGCAAAGTGTCTC (attachment, Figure 1).

Table 2: The Frequency of *Malassezia* in Seborrheic Dermatitis patients with HIV and non-HIV

	PCR POSITIVE	NEGATIVE	TOTAL	P
	4 80.0%	1 20.0%	5 100.0%	
	19 76.0%	6 24.0%	25 100.0%	
Total Count % of total	23 76.7%	7 23.3%	30 100.0%	

**Chi square (X^2) test*

Table 3: Relation of HIV and non-HIV on *Malassezia*

	PCR POSITIVE	NEGATIVE	TOTAL	P
	4 13.3%	1 3.3%	5 16.7%	
	19 63.3%	6 20.0%	25 83.3%	
Total Count % of total	23 76.7%	7 23.3%	30 100.0%	

**Chi square (X^2) test*

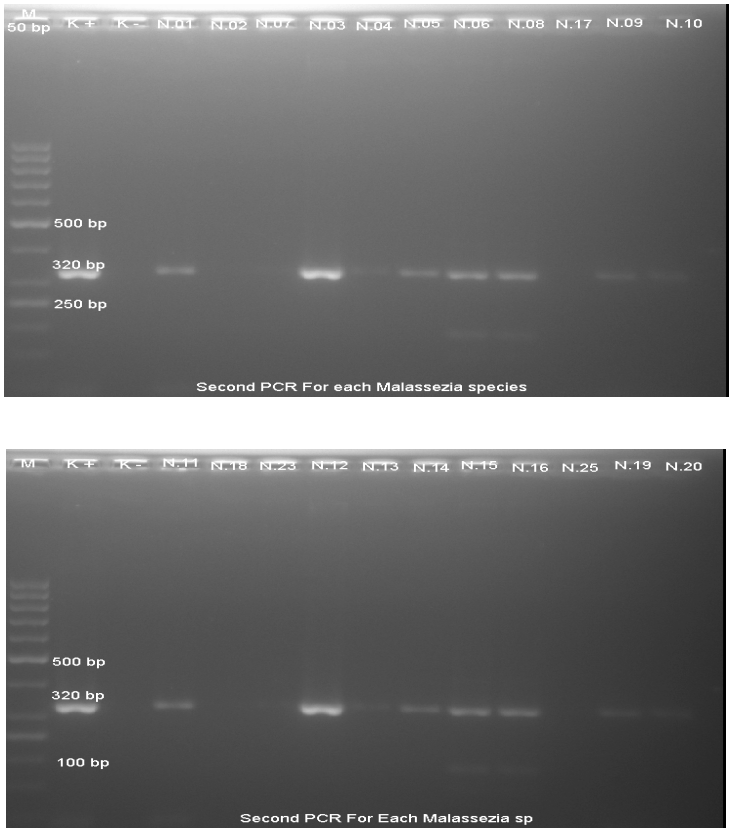

Figure 1: Nested-PCR Results

4. Discussion

This study showed that the frequency of Malassezia species is quite enough in Seborrheic dermatitis patients, of which from 30 samples obtained 23 samples were PCR positive and 7 samples were negative, with Malassezia restricta as dominant species. Of the 5 samples of HIV patients, 4 samples (80%) were PCR positive, while from 25 samples of non-HIV patients, 19 samples (76%) were PCR positive.

Seborrheic dermatitis is a skin condition with sub-acute or chronic inflammation characterized by pruritus, oily erythematous plaque, yellowish-gray scales on areas that rich in sebaceous glands, such as the face, head, upper chest and back. Seborrheic dermatitis affects about 3-5% of adult population, with a tendency to occur in men [1,8].

The cause of seborrheic dermatitis is not yet elucidated, some factors play a role in etiopathogenesis, one of which is the Malassezia species. Several clinical studies have shown increased density of Malassezia has an important role in the pathogenesis of seborrheic dermatitis [3].

The number of subjects in this study were 30 samples of men and women with age ranging from 31-68 years. The age group of 41-70 years had the largest sample size of 20 subjects compared with the 18-40 year age group with a sample of 10 subjects.

Elderly patients have an impaired immune system that is more susceptible to various diseases such as Seborrheic dermatitis. A retrospective study by Malak and his colleagues in Manado, Indonesia reported the highest distribution of seborrheic dermatitis was 45-64 years old age group [9]. Based on this study, the distribution of Seborrheic dermatitis was higher in male than female. The number of male subjects was 17 (56.7%) and women as many as 13 cases (43.3%). This result is similar to previous research conducted by Malak and his colleagues (2016) in Manado who reported 61 subjects (67.0%) were male and 30 subjects (33.0%) were female. This may be associated with higher androgen hormone stimulation in male than in female. Androgen hormones function to produce sebum, and sebum activity is one of the causes of seborrheic dermatitis [9]. Based on the family history of Seborrheic dermatitis, 3 subjects (10.0%) were positive and 27 subjects (90.0%) had no family history. According to the literature, family history is often reported. Recently, there was a mutation of ZNF750 encoding zinc finger protein (C2H2) causing an autosomal dominant seborrhea-like dermatosis, this was found in a family history of Moroccan-Jewish-Israel decent [1]. Seborrheic dermatitis is one of the most common forms of dermatosis in patients with HIV and AIDS [1]. Based on this study, total PCR positive results were 23 samples (76.7%), and negative PCR results were 7 samples (23.3%). *M. restricta* was the dominant species detected in positive samples of both HIV and non-HIV patients. According to the literature, most studies around the world show *M. restricta* as the dominant species in patients with seborrheic dermatitis. Research conducted by Lim and his colleagues using the Nested-PCR method, and Lee and his colleagues using the PCR-RFLP method, identified Malassezia species in Seborrheic dermatitis patients in Korea, with *M. restricta* (47%) and M. furfur (27%) as the dominant species [6,10]. The result of this study is similar with previous researchers. The relation of HIV status to Malassezia species in this study showed positive PCR results in 4 samples (13.3%) of HIV patients and 19 samples (63.3%) of non-HIV patients. Negative PCR results were obtained in 1 sample (3.3%) of HIV patients and 6 samples (20%) of non-HIV patients. 80% of dermatitis seborrheic patients with HIV showed positive PCR results and 1 sample (20%) with negative PCR results. Whereas in seborrheic dermatitis patients with non-HIV, 19 (76%) of them showed positive PCR and the rest of 6 samples (24%) showed negative PCR results. There was no significant difference in the incidence of Malassezia species between HIV and non-HIV patients, with P = 1,000. This is most likely affected by the unequal amount of samples between HIV and non-HIV patients.

Nested-PCR is a fast method compared to other processes such as culture techniques. By using Nested-PCR, if any erroneous fragments were amplified, then it's probable that part will be amplified a second time by a second primer. Thus, nested-PCR is a PCR that has a higher sensitivity and specificity than ordinary PCR in amplifying [7].

5. Conclusion

The researchers concluded that the high incidence of *Malassezia* species was found in patients with seborrheic dermatitis, with *Malassezia restricta* as the dominant species. It is suggested that further research should be conducted with larger sample size. Further research can be performed in a cohort study of the clinical viability of seborrheic dermatitis to Malassezia and to determine the possibility of non-*Malassezia* factors in seborrheic dermatitis, such as analyzing IgG (Immunoglobulin) and IL-10 (Interleukin) expression.

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Competing Interest

The authors declare that they have no competing interests.

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